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Techniques applied to the study of microbial impact on building materials

R. G. Welton[1], M. Ribas Silva[2], Ch. Gaylarde[3], L. K. Herrera[4], X. Anleo[4], N. De Belie[5] and S. Modrý[6]

(1) University of Paisley, Scotland
(2) PPGEC/CT/UFES, Brazil
(3) UFRGS/MIRCEN, Brazil
(4) University of Antioquia, Medellin, Colombia
(5) Ghent University, Belgium
(6) Czech Technical University, Klokner Institute, Prague, Czech Republic

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ABSTRACT

This paper presents various techniques for materials characterisation in relation with the question of microbial impact. Applications examples of some of these techniques are described as well as the respective results obtained by researchers working in this field. Additional readings are also reported.

RÉSUMÉ

Cet article présente diverses techniques de caractérisation des matériaux vues sous l'angle de l'étude des impacts microbiens. Des exemples d'application de certaines d'entre elles sont décrits ainsi que les résultats obtenus par des chercheurs travaillant dans ce domaine. Une bibliographie complémentaire est également fournie.

1 BIOLOGICAL TECHNIQUES

1.1 Sampling with adhesive tape (SAT)

In many circumstances the sampling and monitoring of a building materials biological growth should be performed using a technique that is not only non-contaminatory but also non-destructive to the construction.

The use of adhesive tape, a technique borrowed from clinical mycology, was first bought into the cultural heritage community by Garagni [1]. Sampling is carried out by gently applying a strip of adhesive tape to the surface being studied, which is then removed and placed on sterile glass microscope slides and kept in a sterile box until arrival in the laboratory. The strips are then cut into small pieces and sorted for microscopic and cultural examination.

Light microscopy is carried out by adding a drop of sterile water or other liquid, dependant on technique you wish to use, on the strip and placing a glass slide on the reverse of the tape (to keep it flat during examination). The drop of liquid often expands the microscopic organisms to make it easier to identify each of them under low magnification.

For SEM examination the adhesive tape is placed (face up) on a stub with bioadhesive tape, the sample is then dehydrated via ethanol series and covered with carbon powder. After this simple preparation SEM observations can be made.

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Cultural analysis is performed by introducing the tape into either the solid or liquid medium of your choice, although typically a nutrient rich general medium is used. After a short period of growth it becomes possible to identify many of the organisms present in the community. This is particularly useful for microorganisms (*e.g.*, algae and certain types of bacteria) which can be grown on a specific medium that does not allow the growth of organisms normally found on non-sterile adhesive tape. Unless the tape can be sterilised (a difficult process), many bacteria and fungi will grow on nutrient rich media as contaminants.

1.2 Cell Counts (CC)

It is often necessary to report on the size of a microbial community, and there are various methods for counting the size of a population using microscope methods to give the total number of cells (live + dead), or viable counting methods to enumerate cells capable of reproduction on artificial laboratory media. A couple of the commonest methods are described below. These methods may be used directly on liquid samples, but when the microorganisms are attached to a surface, they must first be removed by a technique such as scraping, swabbing, ultrasonic vibration, etc. For direct cell counts on an opaque surface, a microscopic technique such as electron microscopy or epifluorescence microscopy can be used.

The Helber counting chamber is a slide 2-3mm thick with an area in the centre called a platform, which is surrounded by a ditch. On the top of the slide is a 1mm^2 grid divided into 400 small squares each 0.0025mm^2 in size. When the glass cover slip is placed over the slide, it leaves a clearance of 0.02mm between itself and the grid; thus the volume over each square is 0.00005ml. The specimen is diluted and then a loopful of suspension is placed on the grid area, the cover glass is placed over the slide and this is then examined underneath a microscope. The number of organisms per square is counted until the count reaches 300-500. Dividing the total count by the number of squares examined to reach this number, multiplying by 20 000 000 and the original dilution factor, will result in the number of organisms per ml [2]. This method is more suitable for counting algae or fungal spores than for bacteria, as the X100 objective cannot be used with the thick cover slips required.

In the drop count method, developed by Miles and Misra, small drops (generally 10 or 50µl) of the suspension are placed on agar plates and the colonies in the inoculated areas are counted after incubation. The concentration of cells in the original sample may be calculated as colony forming units per cm^3 (cfu cm^{-3}). This method may also be used for arbitrary standards; *e.g.* if less than ten colonies per five drops of sample the specimen is deemed fit for use.

1.3 Streak tests (ST)

Streak tests are used for two distinct purposes, firstly to isolate pure species from an environmental sample and secondly to test the purity of the isolated organisms. Petri dishes containing a nutrient agar medium are prepared. A flame-sterilised wire loop is used to put a small spot from the sample on the plate. Sterilise the loop again and make a number of parallel streaks through the sample, as shown in Fig. 1. Resterilise the loop and draw a second set of lines perpendicular to the first set, repeat for a third set perpendicular to the second set of lines. Cover and incubate the plate under suitable growth conditions for a period of 24h – 28 days, depending on the growth rate of the specimen.

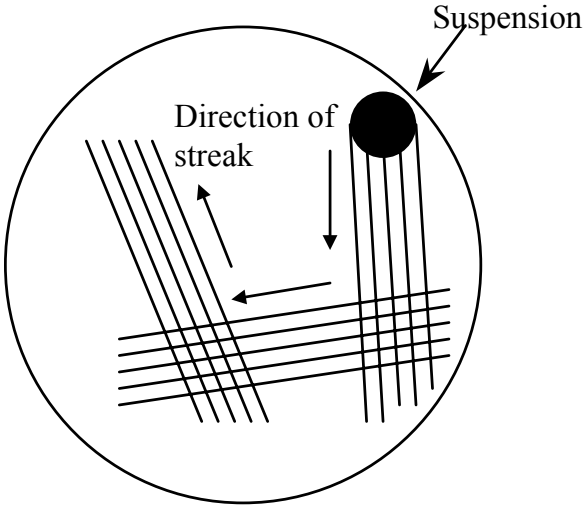

Fig. 1 - The typical streak arrangement on a streak plate.

After incubation, colonies can be examined macroscopically and microscopically to check whether it is a pure sample and to begin the identification process. If further purification is necessary, individual colonies should be picked and streaked repeatedly until identical colonies containing identical cells are obtained.

1.4 Fluorescent cell staining (FCS)

Microbial cells can be stained with a variety of dyes to make them visible under the microscope. For direct examination of cells on an opaque surface, using epi-illumination, fluorescent stains, such as acridine orange and DAPI (4', 6-diamidino-2-phenylindole), may be used. This allows microbial cells to be counted, and their shapes to be distinguished, without removal from the substrate. Cells are, however, normally killed by the staining technique. A variety of fluorescent dyes may be used and some of these allow differentiation of viable, or active, from non-viable, or inactive, cells. Some microorganisms are autofluorescent and can be visualized without staining. These include photosynthetic organisms such as algae and cyanobacteria. There are also ways of making microorganisms autofluorescent, by inserting genes for fluorescence into the cells. Fluorescent green protein is the most common of these and has been used for rapid detection of inoculated organisms in experimental studies to trace the survival and spread of organisms in natural and artificial environments.

1.5 Detection of biomolecules (DB)

Cells may be detected and quantified by analysis of specific biological molecules such as proteins, phospholipids, nucleic acids, chlorophyll and enzymes. These are not necessarily destructive techniques and colour changes

brought about by the activity of respiratory enzymes have been used to quantify microbial biofilms on stone surfaces. However, most of the analytical methods used (for lipids and chlorophyll, for example) necessitate the destruction of the sample, use of suitable extractive chemicals and analysis by chromatographic or spectrophotometric techniques. Enzyme assays may be particularly useful, as they measure active cells, rather than merely cells capable of reproduction to give colonies on solid media.

1.6 DNA analytical methods (DNA-am)

The use of specific nucleic acid probes and the polymerase chain reaction (PCR), which allows the production of multiple copies of selected regions of the cell genome, has only recently begun to be applied to building materials. These are specialised techniques that allow microorganisms to be detected and identified either *in-situ* or *ex-situ* with a high degree of specificity and sensitivity. They are rapid, especially in comparison with culture methods, and allow the detection of microorganisms that cannot yet be grown in artificial culture media. Most methods do not allow ready quantification of the microbial population, although this is possible with FISH (fluorescence *in situ* hybridisation) techniques, which use fluorescent gene probes to visualise microorganisms under the microscope. At present, there are no standard techniques and protocols must be developed for the material under investigation, based on methods already published for clinical and environmental specimens.

1.7 Immunological Methods (IM)

Antibodies labelled with detector molecules such as fluorescent dyes or enzymes can be used to detect and quantify specific microorganisms. These methods have been long used in the medical field, but are relatively rare in building materials research. They seem to offer no advantages over the DNA-based techniques, which are becoming so popular.

2 TECHNIQUES APPLICATION

The research using fifteen of the described techniques are presented by some researchers working in the field of the microbial impact on building materials. Each example of application gives the analysed material, the objective of the study, the results obtained, the conclusion and the advantage of the use of the technique(s). Isolated employment of ten techniques and the use of an ensemble of seven techniques are presented.

2.1 Environmental scanning electron microscope (SEM/ESEM) and energy dispersive x-ray analysis (EDX) - Application example

This technique was applied by the team of Nele De Belie at the Magnel Laboratory for Concrete Research, Dept. of Structural Engineering, Ghent University, Belgium.

Concrete, mortar, limestone were the analysed materials.

The objective of the use of these techniques was to determine the presence of micro-organismes on concrete/stone and to get a first idea about the type of micro-organismes; to visualise the formation of crystals, *e.g.* gypsum or ettringite formed by the action of sulphuric acid excreted by Thiobacilli; to visualise the effect of excreted acids on the cement paste and the transition zone with the aggregates; EDX can be used together with a study of the morpholgy to determine which crystals are present.

The following results were obtained:

- The presence of Thiobacilli on concrete sewer pipes was confirmed (Fig. 2)
- Lichens were observed on concrete (Fig. 3).
- SEM-EDX prove that gypsum crystals are formed on concrete due to sulphuric acid excretion by Thiobacilli (Figs. 4 and 5)
- The presence of ettringite crystals was detected through this technique.
- In a research on microbiologically induced calcium carbonate precipitation for repair of concrete and stone surfaces (biomineralisation), the quality of the deposited $CaCO_3$-layer is studied with SEM.

Details on this research can be obtained in some

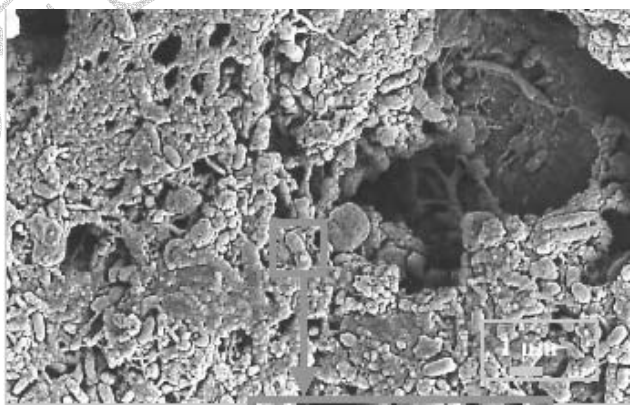

Fig. 2 - *Thiobacillus* on concrete.

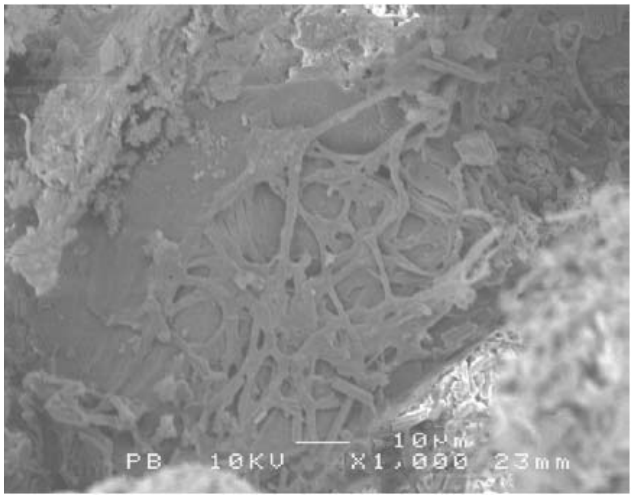

Fig. 3 - Lichens on concrete.

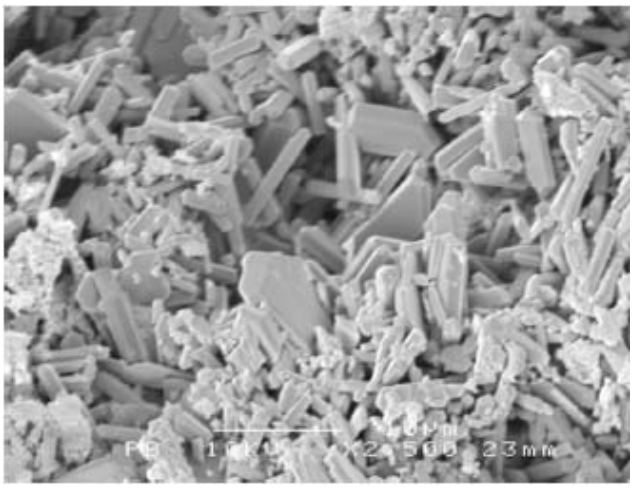

Fig. 4 - Gypsum crystals formed on concrete due to sulphuric acid excretion by *Thiobacillus*.

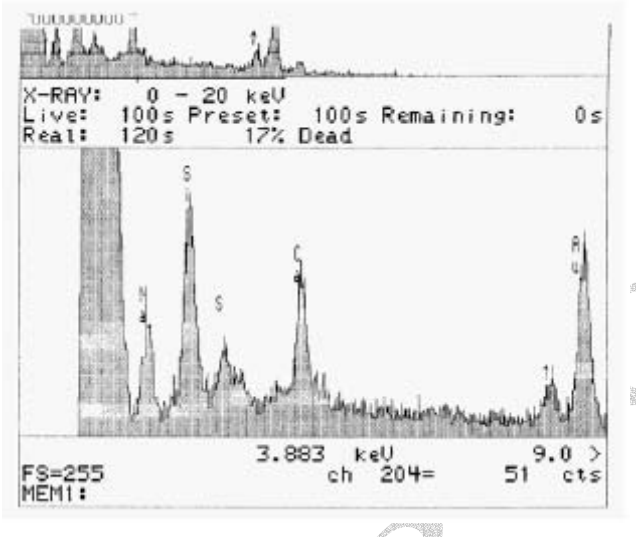

Fig. 5 - EDX confirms the presence of gypsum crystals (S-peak).

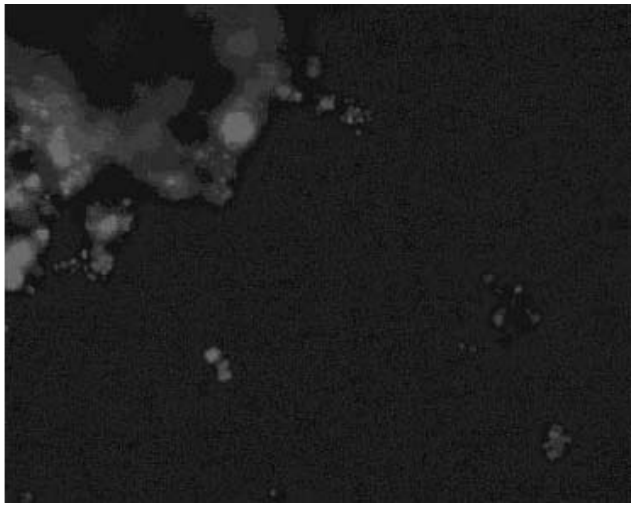

Fig. 6 - Lichens in a fouled concrete surface (red stains). There is no indication of dead bacteria.

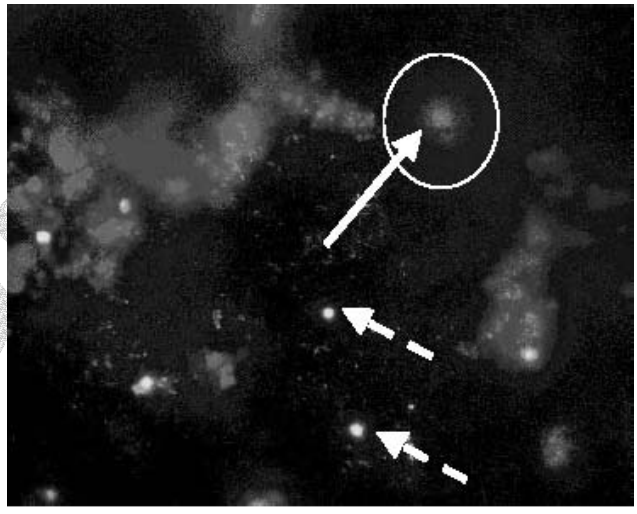

Fig. 7 - The same surface as Fig. 6, with the dead lichens at the same location. In between the fouling, green and yellow dots and vails are clearly visible (white arrow and circle. These are bacteria and biofilms. The big yellow stains are living lichens (white dashed arrows).

references [3-5].

An advantage of this technique is that although a rather small area of the concrete/stone surface is studied, a qualitative impression can be obtained about the presence of micro-organisms and their effect on the substrate.

2.2 Fluorescent cell staining (FCS) - Application example

The team of Nele De Belie carried out this research at the Magnel Laboratory for Concrete Research, Dept. of Structural Engineering and the Laboratory for MicrobialEcology and Technology, Ghent University, Belgium

The analysed materials are concrete and stone.

The objective of the study is to determine presence and viability of micro-organisms in the material.

Viability staining was performed using commercial live/dead stain (L-13152, Molecular Probes, Leiden, The Netherlands). This stain allows fluorescence microscopy to distinguish between organisms with intact cell membranes (stained green and scored alive) and organisms with damaged cell membranes (stained red and scored dead). 25 µl stain was put directly on 1 cm^2 of mortar surface and was incubated for 10 min in the dark and examined by standard epifluorescence microscopy on a Zeiss Axioskop II microscope (Carl Zeiss, Jena, Germany). The microscope was equipped with a Peltier cooled single chip digital colour CCD camera (Hamamatsu Orca IIIm, Hamamatsu, Massy Cedex, France) and connected to a PC to obtain digital images. For each treatment, two preparations were examined under fluorescent microscopy.

Staining of untreated concrete cubes polluted with organic fouling, resulted in a red staining of the dead lichen cells, illustrated in Fig. 6. Clusters of live cells could be detected on the mortar cubes treated with the cleaning Thio-S culture, that were not seen on untreated specimens. These cells were present as groups of attached individual cells (Fig. 7) and also as organised in biofilm structures. It was

proposed that these cells represented active *Thiobacillus sp.* cells from the Thio-S culture. The results are similar for portland and blast furnace slag cement samples. These results prove that the micro-organisms are using the concrete as a substrate and that they can locally produce sulphuric acid, having an active cleaning effect.

The advantage on using this technique is to allow the determination of the presence and viability of micro-organisms. Details on this research can be found in [3].

2.3 Mössbauer spectrometry - Application example

The authors of this study are Prof. Liz Karen Herrera and X. Anleo, both from the University of Medellin, Colombia.

Peridotite, source and weathered rocks of the church of Veracuz in Medellin Colômbia were studied in this research.

Mössbauer analysis was performed to complete the identification of the oxidations products, already made by using FT-IR and XRD [6-9].

The room temperature Mössbauer spectra (MS) were obtained in transmission mode using a constant acceleration drive and triangular reference signal. A 57A Co/Rh source with initial activity of 25mCi was used. The spectra were fitted using a program called MOSF which is based on a non-linear least squares fitting procedure which assumes Mössbauer lines of Lorentzian shape standard hematite was used to calibrate regularly the spectrometer and the calibration line width was about 0.28 ± 0.02 mm/s.

Comparing the Mössbauer spectrum of source rock (Fig. 8) with that of weathered rock (Fig. 9), the two doublets in Fig. 8 should correspond to olivines whereas the doublest of weathered rock in Fig. 9 should be assigned to the present of source rock, (the major one) and the to a mixture of goethite and lepidocrocite (as a result of weathering processes).

MS provides the complete, *in situ* measurement, non-destructive, three-dimensional identification of corrosion products and is the only technique able to accurately measure the fraction of each oxide in a corrosion product layer. Thus, it can be used to study the electric, magnetic and structural characteristics of metals, alloys, soils and minerals. MS can be considered as a "fingerprint" technique of varying degrees of sophistication in mineralogical and geo-chemical studies. More fingerprint applications are characterization of oxidation state iron (*e.g.*, Fe^{2+} or Fe^{3+}), electronic configuration of iron (*e.g.* high or low spin) coordination symmetry about the iron atom (*e.g.* octahedral or tetrahedral) and site distortion from either octahedral or tetrahedral symmetry.

2.4 Optical microscopy - Thin section petrography - Application example

Nele De Belie, from the Magnel Laboratory for Concrete Research, Dept. of Structural Engineering, Ghent University, Belgium, developed a study using this technique.

Concrete, mortar and limestone were analysed.

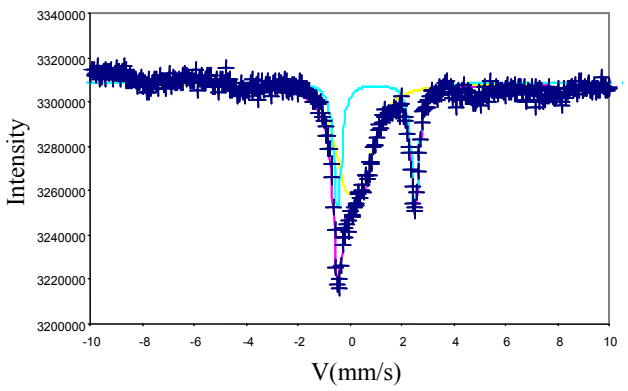

Fig. 8 - Mossbauer spectrum of peridotite (source rock). Source rock of the church of Veracuz in Medellin Colombia.

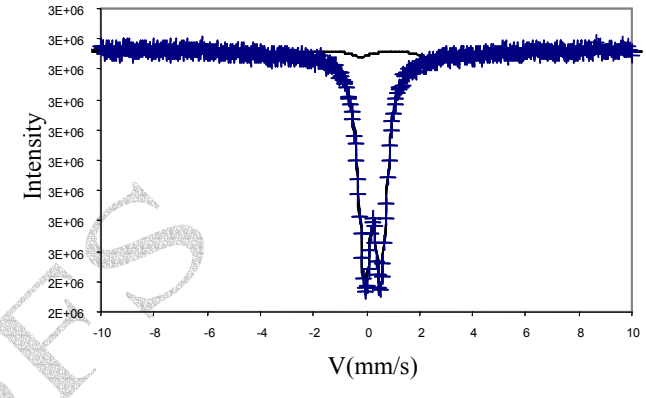

Fig. 9 - Mossbauer spectrum of peridotite (weathered rock). Weathered rock of the church of Veracuz in Medellin Colombia.

The aim of the study was to determine the degradation depth of the specimens; to visualise to which extent the material is influenced for instance by acids excreted by bacteria; to visualise concrete carbonation; to study the presence of porosities or microcracking, suggesting the action of acids or expansive salts, respectively. For the study of pores and cracks, the fluorescence mode can be used besides the classical ordinary and polarised light modes. To allow use of fluorescence microscopy, the samples should be impregnated beforehand with an epoxy resin containing a fluorescent dye.

See for instance Fig. 10: the same thin section is photographed at ordinary light (top), polarised light (middle) and fluorescent light (bottom). The presence of a more porous zone at the edge of the specimen is clearly visible (brown coloration at polarised light and more intense fluorescence at fluorescent light). This zone has been degraded by lactic acid, extreted by bacteria.

From the border to the core of concrete samples degraded by lactic acid, three zones could be distinguished with different stage of degradation. The samples with blast furnace slag cement had a denser structure than the samples with ordinary Portland cement (OPC), with less air voids and a smaller capillary porosity. Also test specimens with limestone aggregates seemed to have a smaller capillary porosity than the corresponding specimens with gravel aggregates. In the outer concrete layer, the transition zone between cement paste

and aggregates had been highly degraded, especially for the concretes with OPC.

More details on this research can be found in [10]. These techniques are very useful to quantify how deep the effect of the bacteria is penetrating into the concrete or stone specimens.

2.5 Mercury intrusion porosimetry (MIP) - Application example

This technique has been used by Sylva Modrý from the Czech Technical University, Klokner Institute, Prague, Czech Republic.

Cementitious materials - pastes, mortars, concrete, rocks (also weathered), ceramics, catalysts supports, wood, paper, insulations, sintered powder iron and corroded concretes were analysed [11].

Mercury is intruded into pore structure of materials under high pressure. From the dependence of intruded volume of mercury on the pressure applied pore structural characteristics can be assessed.

Specimens for MIP measurement are usually prepared as granules (*e.g.* of 3-5 mm in diameter). All liquids must be removed from the specimens before mercury penetration. In the case of hardened binders the mode of drying plays extremely important role, as was proved by D. Winslow and S. Diamond [12].

As relatively high pressures are used in MIP it is of utmost importance to know whether and to what extent the samples under investigation are affected. Permanent changes, *i.e.* plastic deformations and/or deterioration can be pronounced in some materials that corrections have to be introduced with respect to compressibility. In some cases the method cannot be used at all, *e.g.* some coals or sandstones may serve as an example. From the results given in the literature follows that resp. distortion of samples is dependent on mechanical properties of the solid phase itself, and at the same time on the shape and size of the pores and on the mode of their mutual connection. In general, materials with narrow pore entries leading to large cavities are less resistant and, consequently, easier to deform [13]. The used value of the pressure plays a great role. For example pressures up to 98 MPa do not cause measurable alteration in the sample of hardened cement [14].

The Washburn model is used to convert mercury intrusion data under two assumptions: 1) the pores are cylindrical and 2) that they are accessible to the outer surface of specimens. It is obvious that the pore shapes in majority of real natural or artificial materials are quite different from cylindrical pores. This fact influences the output of MIP pore size distribution measurements. The obtained sizes of pores are much smaller than their actual sizes. This can be proved by comparison of results of an indirect MIP method with a direct microscopical observation [15].

Due to the problems listed above, Diamond [16] recommends to use the threshold diameter and the total volume of intruded mercury only as parameters for comparison with pore structure of other cement pastes or cement mortars, instead of plotting MIP size distribution according to the Washburn model, which does not reflex the actual distribution of pore sizes.

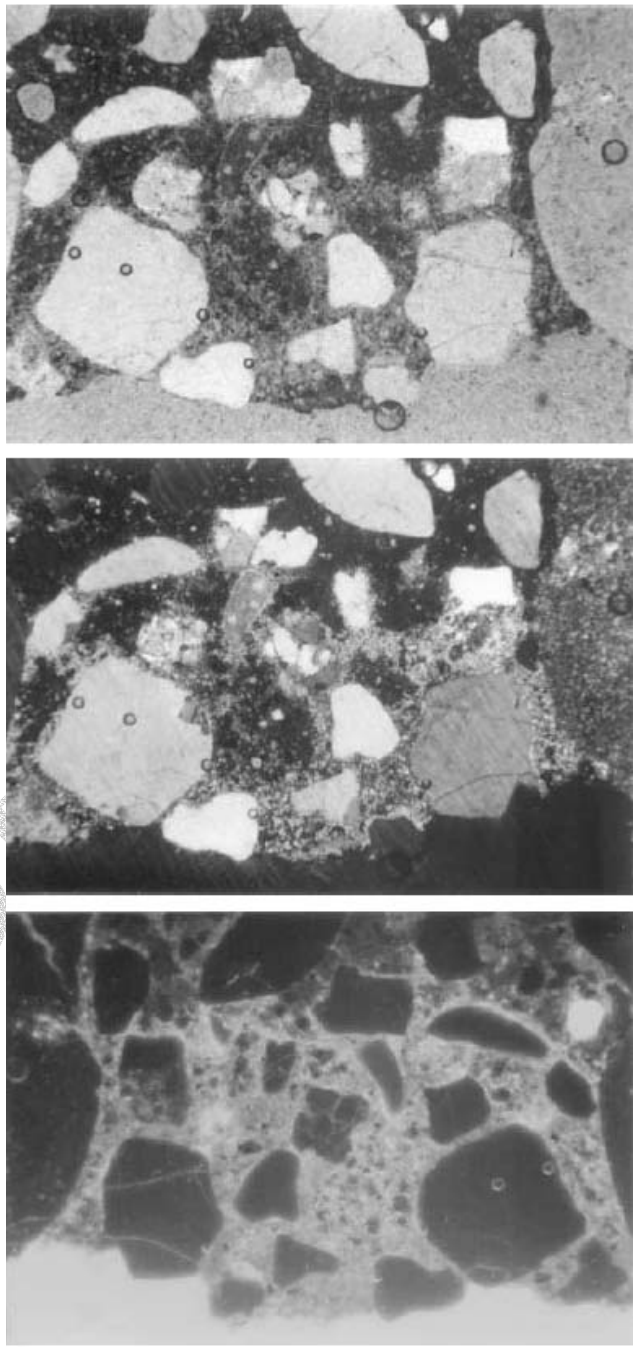

Fig. 10 - Thin sections photographed at ordinary light (top), polarised light (middle) and fluorescent light (bottom).

The grade of complexity of the pore system can be qualitatively assessed with the aid of retention coefficient, which shows what the volume of the mercury trapped in the pore system is. The mercury penetration and retraction curves for hardened cement pastes with different ratio of cement to water can be seen in the Fig. 11 [17]. The dependence of the retention coefficient on water to cement ratio and also on porosity can be seen.

The advantage of MIP method is that it is very simple and fast.

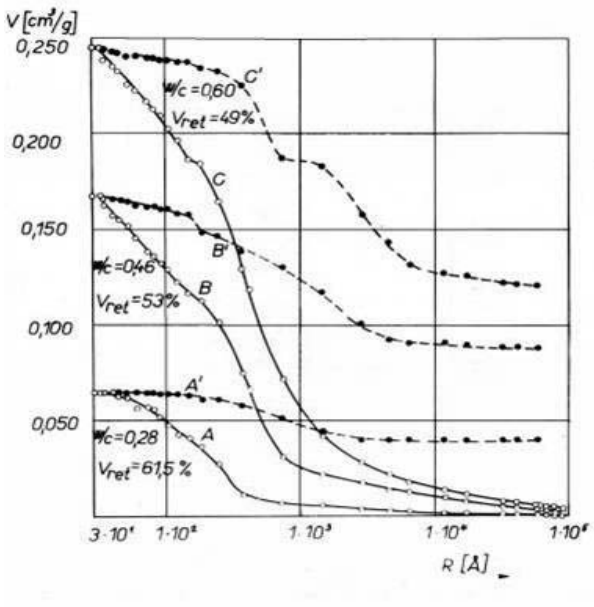

Fig. 11 - Penetration (A, B, C) and retraction (A', B', C') curves of dependence of forced in mercury volume V on pore radius R.

Main disadvantage is the application of high pressure. It is necessary to find what the behaviour of material tested under high pressure is.

2.6 Water uptake - Application example

The team of Nele De Belie used this technique at the Magnel Laboratory for Concrete Research, Dept. of Structural Engineering, Ghent University, Belgium in collaboration with the Laboratory for Microbial Ecology and Technology, Ghent University.

Water absorption is the process whereby fluid is drawn into a porous unsaturated material under the action of capillary forces. The capillary suction depends on the pore volume and geometry, and the saturation level of the stone. Water absorption is an important transport mechanism near the surface, and can therefore be related to the durability of the surface layer.

A modified version of the sorptivity test based on the Belgian standard NBN B 05-201 was used to determine the effect of a microbiologically produced calcium carbonate layer on the water absorption of concrete or stone. The specimens are coated at the four edges adjacent to the side treated with the microbiological suspension, to ensure unidirectional absorption through the treaded side.

Capillary water absorption is compared to full saturation under vacuum.

The effect of a microbiologically induced calcium carbonate layer on the water absorption could be monitored. This allowed to select specific strains of Bacillus sphaericus that were effective to precipitate a regular calcite layer and to decrease capillary water uptake. The effect of the precipitated layer on Euville limestone was concentrated on the first 2 days of capillary water uptake. After 2 days all the tested samples reached more or less the same saturation level [4, 5].

2.7 Weight loss - Application example

The use of this technique was applied by Nele De Belie from the Magnel Laboratory for Concrete Research, Dept. of Structural Engineering, Ghent University, Belgium

The objective of the research was the measurement of weight loss of concrete or stone samples in a laboratory test on microbial (or chemical or mechanical) deterioration is a very easy and direct method to quantify the deterioration.

The bioreceptivity of different concrete types or stone specimens can be monitored. For instance the degradation of concrete types with different aggregates (limestone/gravel), production methods (immediate form removal/hardening in the formwork/centrifugation) or cement types (portland cement/blastfurnace slag cement) by biogenic sulfuric acid corrosion was obtained [18].

The results of the higher mentioned study are:

Concrete with limestone aggregates showed a smaller degradation depth and weight loss than concrete with inert aggregates. The limestone aggregates locally created a buffering environment protecting the cement paste. This was confirmed by microscopic analysis of the eroded surfaces. The production method of concrete pipes influenced durability through its effect on W/C ratio and water absorption values. In the microbiological tests, HSR Portland cement concrete performed slightly better than slag cement concrete. A possible explanation can be a more rapid colonisation by micro-organisms of the surface of slag cement samples.

The advantage of the method is that it is a very simple and direct measurement method.

2.8 X-ray diffraction analysis - Application example

Nele De Belie applied this technique on studies carried out at the Magnel Laboratory for Concrete Research, Dept. of Structural Engineering, Ghent University, Belgium.

The analysed materials were concrete, mortar, stone.

The aim of the study was to determine the composition of crystalline compounds formed through microbial action.

E.g. Fig. 12 shows crystals that were formed on concrete

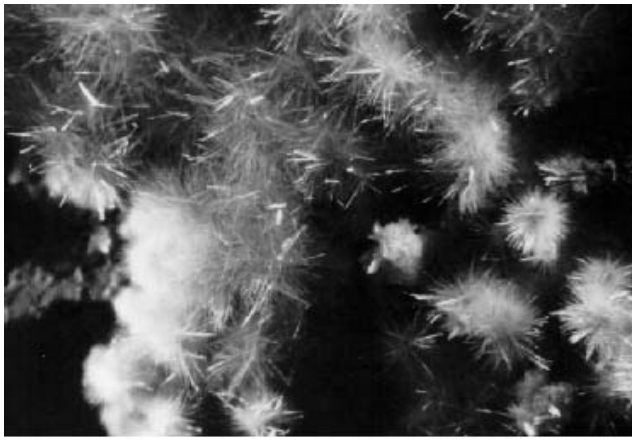

Fig. 12 - Crystals formed on concrete through action of bacterially produced organic acids (lactic and acectic acids).

specimens through interaction with bacterially produced organic acids (lactic and acetic acid). XRD revealed that these crystals contained calcium acetate, different calcium acetate hydrates and calcium lactate hydrate.

Also calcium carbonate crystals formed through microbiological precipitation by Bacillus sphaericus were investigated with XRD: the precipitated calcium carbonate appeared to be calcite and vaterite crystals [5, 19].

The technique allows obtaining the mineralogical composition of degradation products.

2.9 Set of six techniques (CA, TG, DTA, XRD, SEM and MC) - Application example

Research carried out by Moema Ribas Silva (from the PPGEC, Federal University of Espírito Santo - Vitória, Brazil) and her group, at the University of Brasília. They used six different techniques in order to analyse concrete samples taken from structures submitted to three different climates.

The following techniques were applied together for studying the biodeterioration of concrete: Chemical Analysis (CA), Thermogravimetry (TG), Differential thermal analysis (DTA), X-rays diffraction (XRD), Scanning electron microscopy/energy dispersive analysis (SEM/EDX) and Mineralogical calculation (MC).

These techniques give useful information on the material condition concerning its deterioration (biodeterioration). DTA and XRD identify the amorphous and crystallised compounds, including the deterioration products. SEM / EDX allows observing, among others, the presence of these compounds, the texture of the material as well as detecting the presence of microorganisms and characterising morphologically these microorganisms. With the aid of the MC (which uses the results of CA and TG as data), it is possible to identify the deterioration (or biodeterioration) mechanisms what allows to indicate a method for stopping or avoiding it. A study on the influence of three different environmental conditions on the concrete biodeterioration was carried out.

Samples were collected on damaged concrete structures submitted to three different environmental conditions (temperature from –5°C to 35°C and relative humidity from 8 to 100%), aged of 7 to 30 years [20-22]. Even if these conditions were quite different, microorganisms were present in the concrete submitted to the three studied climates. The same concrete compounds, among others micas, feldspars, quartz, calcic compounds (Fig. 13), appeared solubilised in the concrete from the three analised climates. Besides the same kind of microorganisms, such as diatom algae (Fig. 14), Actinomycete, *Thiobacillus, Cladosporium*, Protozoa (Fig. 15) etc., some different unidentified morphologies (Fig. 16) were observed in the studied concrete structures.

Fig. 13 - Solubilised calcium carbonates observed in a concrete where Cladosporium and protozoa were also present.

Fig. 14 - Diatom algae found in the same sample as Fig. 9 taken from a concrete structure placed in a hot and dry environment.

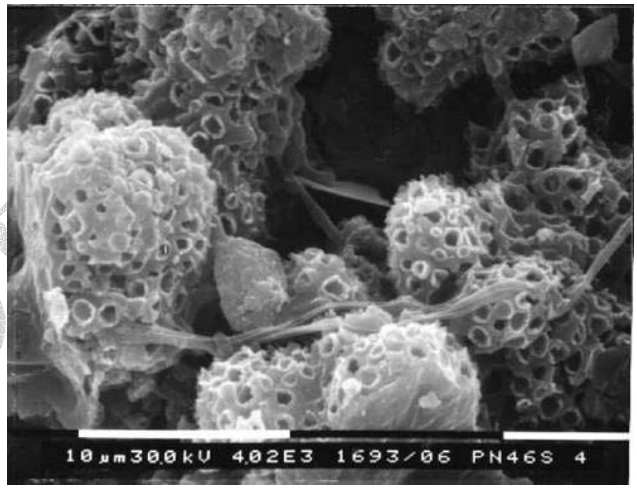

Fig. 15 - Unidentified morphology close to fungus hyphae. Organic matter was detected by ,+differential thermal analysis in this sample.

Through mineralogical calculation it was possible to verify that part of the silica was missed when diatom algae (which uses silica as a nutrient) was present in the concrete.

Deterioration products, such as ettringite (Fig. 17) and gypsum (among other sulphates) were detected in the analysed concrete structures but the sulphur source could not be detected.

This study leads to the following conclusions:

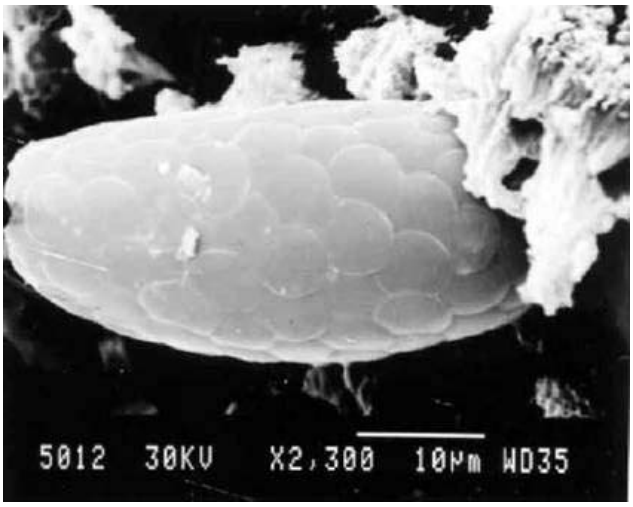

Fig. 16 - Protozoan found in the concrete submitte do a cold and humid climate.

Fig. 17 - Ettringite observed in a concrete appearently sound but where it was observed deteriorated micasand solubilised calcium carbonates and portlandite.

- The concrete biodeterioration can be developed under different normal climates conditions (extreme temperature and relative humidity were not considered) independently from the environmental conditions. So they are not a very important factor on the development of microorganisms.
- Some of the morphologically characterised microorganisms were the same in the concrete under the three analysed environmental conditions, however those uncharacterised forms are quite different.
- The mentioned set of techniques is very useful, when used together, on the study of concrete biodeterioration, however they should be completed with the use of microbiological tests in order to identify microorganisms present in the concrete and the corresponding metabolisms, even if these techniques allow detecting some of the biodeterioration mechanisms. The most frequently observed mechanisms were the silica solubilisation by diatom algae and the calcium compounds solubilisation by acids produced by certain microorganisms such as fungi and bacteria.
- SEM/EDX was considered the main technique of this study but it is necessary to use other techniques in order to get all the necessary results for taking a coherent conclusion for completeni the study.

2.10 DNA analytical methods

Vincke and his team used conventional as well as molecular techniques to determine the microbial communities present on the concrete walls of sewer pipes [23]. The genetic fingerprint of the microbiota on corroded concrete sewer pipes was obtained by means of denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments. The DGGE profiles of the bacterial communities present on the concrete surface changed as observed by shifts occurring at the level of the dominance of bands from non-corroded places to the most severely corroded places. By means of statistical tools, it was possible to distinguish two different groups, corresponding to the microbial communities on corroded and non-corroded surfaces, respectively. Characterization of the microbial communities indicated that the sequences of typical bands showed the highest level of identity to sequences from the bacterial strains *Thiobacillus thiooxidans*, *Acidithiobacillus* sp., *Mycobacterium* sp. and different heterotrophs belonging to the a-, ß- and γ- *Proteobacteria*, *Acidobacteria* and *Actinobacteria*. In addition, the presence of *N*-acyl-homoserine lactone signal molecules was shown by two bio-assays of the biofilm on the concrete under the water level and at the most severely corroded places on the concrete surface of the sewer pipe.

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Appendix: ADDITIONAL READINGS

a) Sampling with adhesive tape

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